# Lysostaphin

### Week 16

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

## Summary

# 1 Biofilm assay of pSCB1C3-T7-Lys: Inhibition

## Responsible

Shuangjia Xue, Jenny Waspe, and Aliki Mitropoulou

## Protocols used

Biofilm Assay

# Experimental Set Up

At first we performed protein extraction of Lysostaphin where the cells were lysed by sonication and after centrifugation, both the cell pellet and the supernatants were tested for biofilm inhibition. The supernatant samples were labelled Lys (S), whereas the pellet samples were labelled Lys (P). The treatment had been done on 3 different samples of Lys (Lys 1, Lys 2 and Lys 3) but for simplicity, only one is shown in each experiment.
For the inhibition test, the experimental procedure is first done by adding liquid cultures of P. aeruginosa or S. aureus which we immediately treat with Lysostaphin at four different volumes; 1, 5, 10 and 20 l. The treated biofilm was left to incubate for 48 hours and after that the biofilm was stained with crystal violet 0.1 percent. The absorbance of the crystal violet was measured at 595 nm. Gentamicin was used as positive control and cell lysates of BL21 cells as negative control.

# Results and Conclusions

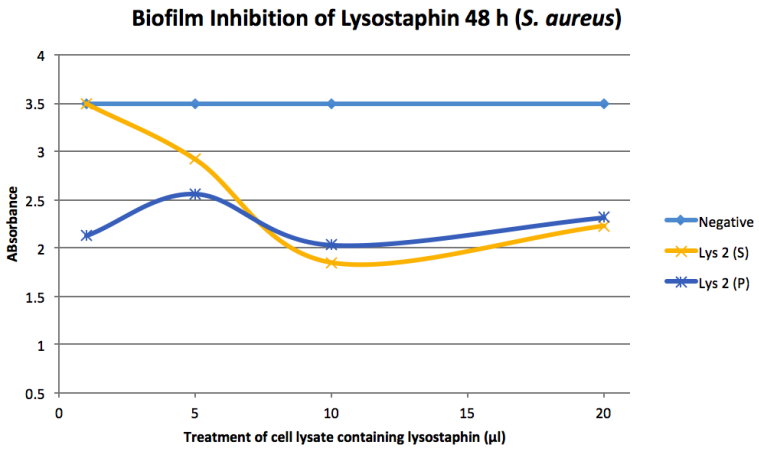


Figure 1: Absorbance measured at different volumes cell lysate treatment containing Lysostaphin using *S. aureus* as biofilm-producer. Negative: cell lysates of untransformed BL21 cells. Lys 2 (S): Cell lysate containing lysostaphin from the supernatant fraction. Lys 2 (P): Cell lysate containing lysostaphin from the pellet fraction.

This graph indicates that the absorbance reading is generally reduced with increased volume of Lysostaphin treatment. The graph also shows that the drop in absorbance, which indicates a lower degree of crystal violet present in the wells, is prominent in both the supernatant and pellet sample. This reflects the SDS PAGE results that show the presence of Lysostaphin is in both the supernatant and the pellet. The constant negative control is maintained at 3.5 absorbance. Therefore t-test is viable to determine the statistical significance of the results. From the t-test it is observed that there are significant differences in biofilm inhibition for the samples Lys 2 (S) with a P-value less than 0.05. A significance value was also observed for Lys 2 (P) at 5 and 10 ul with a a P-value less than 0.001. The positive control which used gentamycin had a much lower average absorbance of 1.065.

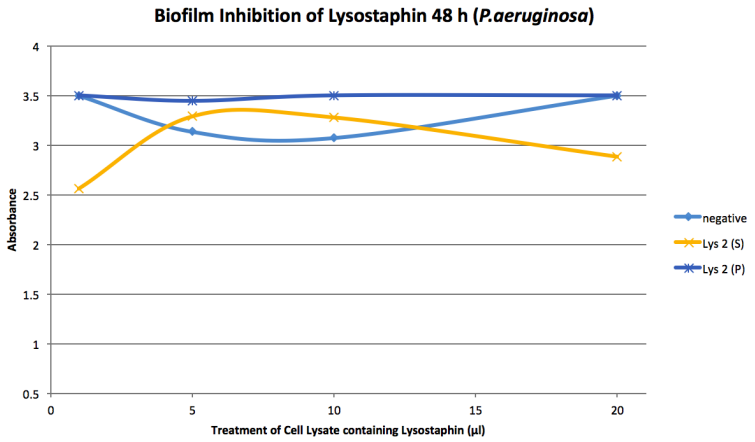


Figure 2: Absorbance measured at different volumes cell lysate treatment containing Lysostaphin using *P. aeruginosa* as biofilm-producer. Negative: cell lysates of untransformed BL21 cells. Lys 2 (S): Cell lysate containing lysostaphin from the supernatant fraction. Lys 2 (P): Cell lysate containing lysostaphin from the pellet fraction.

Unlike the significant inhibitory results on *S. aureus*, data on *P. aeruginosa* was mostly insignificant, as shown from the figure above. Based on this figure, we can say that the changes in absorbance from the treatment was insignificant compared to the negative control. This was expected as we knew that lysostaphin specifically targets the peptidoglycan layers in staphylococci.

# 2 Biofilm assay on pSCB1C3-T7-Lys: Dispersal

## Responsible

Shuangjia Xue, Jenny Waspe, and Aliki Mitropoulou

## Protocols used

Biofilm Assay

# Experimental Set Up

Lysostaphin was expressed and cells were lysed by sonication to extract protein. In the following centrifugation step, both the cell pellet and the supernatants were kept for the biofilm dispersal. The supernatant samples were labelled Lys (S) for supernatant and Lys (P) for pellet samples. The treatment had been done on 3 different samples of Lys (Lys 1, Lys 2 and Lys 3) but for simplicity, only one is shown in each experiment.
For the inhibition test, the experimental procedure is first done by adding liquid cultures of P. aeruginosa or S. aureus which we left to incubate for 48 hours. After the biofilm had been established, treatment of cell lysates containing lysostaphin was observed at at four different volumes; 1, 5, 10 and 20 ul. After that the treated biofilm was stained with crystal violet 0.1 percent. The absorbance was measured at 595 nm. Gentamicin was used as positive control and cell lysates of BL21 cells as negative control.

# Results and Conclusions

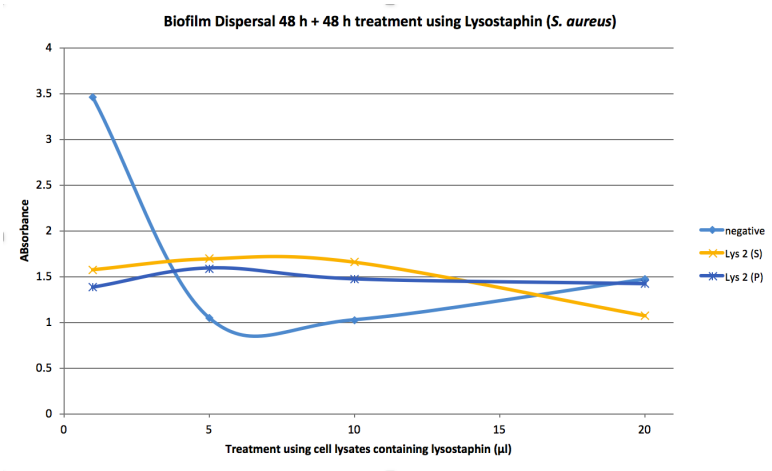


Figure 3: Absorbance measured for different treatments of Lysostaphin using *S. aureus* as biofilm-producer. Negative: cell lysates of untransformed BL21 cells. Lys 2 (S): Cell lysate containing lysostaphin from the supernatant fraction Lys 2 (P): Cell lysate containing lysostaphin from the pellet fraction.

The graphical presentation of results clearly shows that the negative control has reducing absorbance with increased treatment, although it should be constant. This steep decrease in the

negative control sample could indicate that other proteins from the cell lysate may play a role in causing the dispersal of *S. aureus* biofilm.
In contrast, the treated wells with lysostaphin samples show a reduced absorbance that remained constant with increased volume of treatment. In the treated samples we see that at low treatment volumes the absorbance is already low, at around 1.5. This is comparable to that of the positive control which has an average absorbance of 1.09 (not shown). Hence, even though we do not see a declining slope of absorbance towards an increase of treatment volume, it was likely that with low volumes, lysostaphin was able to disperse *S. aureus* biofilm. This is confirmed by the t-test where there a significant effect of biofilm dispersal at a P-value ¡0.03 for a majority of the Lys 2 samples.

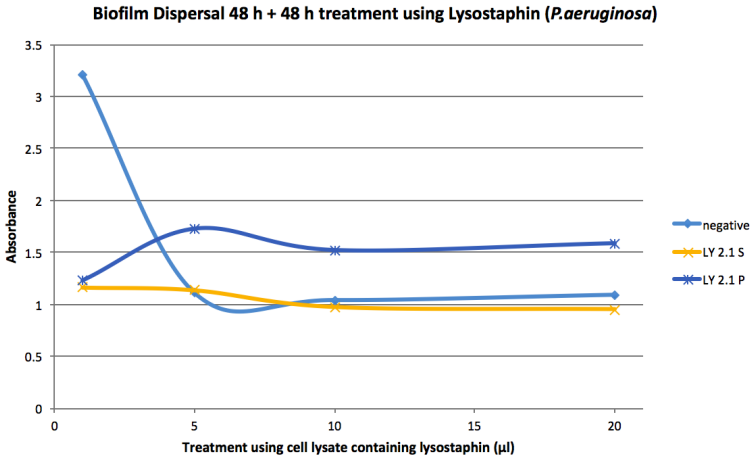


Figure 4: Absorbance measured for different treatments of Lysostaphin using *P. aeruginosa* as biofilm-producer. Negative: cell lysates of untransformed BL21 cells. Lys 2 (S): Cell lysate containing lysostaphin from the supernatant fraction Lys 2 (P): Cell lysate containing lysostaphin from the pellet fraction.

The graphical illustration of dispersal results of *P.aeruginosa* is similar to that of inhibition results. Firstly, we see that there is an unexpected drop in the negative control. Although it shows that there are relatively constant absorbances observed for the wells treated with Lysostaphin, each sample have dispersed it to different degrees. The t-test shows that for most of the treated samples, the result was insignificant which indicates that lysostaphin is ineffective against P. aeruginosa biofilm dispersal.
The dispersal of biofilm assay show some sample treatments to significantly reduce optical density for biofilms produced by *S. aureus*. The data shows an invalid negative control that shows a decrease in the biofilm OD, which may be due to cell debris inhibiting biofilm formation. There is however a clear pattern in the samples treated that affects the biofilm at a constant level throughout the increased volume of treatment. But there is no clear difference between the supernatant and pellet samples, that was have seen in the inhibition results. Increased volume of treatment (20l, 50l) showed a clear inhibition of biofilm formation for *S. aureus* but not for P.aeruginosa. To improve the quality of data obtained, more replicates are needed to get valid results.